VARIATION AND INHERITANCE IN SIZE IN TRYPANOSOMA LEWISI

II. THE EFFECTS OF GROWING "PURE LINES"[1] IN DIFFERENT VERTEBRATE AND INVERTEBRATE HOSTS AND A STUDY OF SIZE AND VARIATION IN INFECTIONS OCCURRING IN NATURE

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In our first report[2] we demonstrated that Trypanosoma lewisi reaches an adult stage in the course of the infection in the rat in about 25 days, and once this stage is reached there is practically no division or growth. In consequence of the elimination of growth factors from the computations it was found that the coefficient of variation in the "pure line" was very low if it was computed during this adult period. The questions which now arise are: (1) Does growing the same "pure line" in different vertebrate hosts cause significant differences in the mean or the coefficient of variation? (2) Does passage of the "pure line" through the invertebrate host cause any significant differences in these constants? Our attention will be directed especially toward the coefficient of variation as this will give us an opportunity to ascertain whether or not there is a splitting up of the "pure line" into heritably diverse lines following such passage through the invertebrate host. With this background our final question is: (3) Does an infection occurring in nature consist of a large number of "pure lines" such as have been described in free-living protozoa, which differ among themselves but are *per se* constant in size?

It is to be emphasized at this point that in all of the following work the measurements and computations were made on or after the 30th day of the blood infection. As the adult stage is always reached by the 25th day this is well into the adult period and effectually eliminates growth factors. The six measurements of size used in this work have already been given in our first report (see figure 1). They consist of the following distances: (1) Posterior end *to* parabasal body, (2) parabasal body *to* nucleus, (3) nucleus *to* anterior end, (4) anterior end *to* end of flagellum, (5) total length which includes measurements 1–4, and (6) width.

Effect of Growing the Same "pure line" in Different Vertebrate Hosts.— The twoconstants in which we are interested in this connection are the size as indicated by the mean and the variability as indicated by the coefficient of variation. In all of the experiments the constants were worked out for all six measurements but in this brief report we will only deal with those for total length. Let us first consider the mean. The questions are: Does growing the same pure line in different *individuals* of the same

species of rat produce significant differences in size? Does growing the same "pure line" in different *species* of rats produce any greater differences in size than in the first case of individuals belonging to the same species? It would have been interesting to grow the "pure line" in other vertebrates as well as in rats but T. lewisi is not normally infective to any other vertebrate. To test the first of these questions we used individuals of the albino rat and for the second question we used individuals of the black and the Norway rat. At the outset it may be definitely said that the differences which were obtained on growing the "pure line" in different species of rats were in no way more significant than the differences obtained by growing the "pure line" in different individuals of the same species. The greatest difference in mean size[3] was obtained when the same "pure line" was grown in the albino rats 46 and 90. In the former the mean length was $32.080 \pm .052$ and in the latter $30.769 \pm .057$, which gives us a difference of $1.311 \pm .077$. The mean lengths obtained in twelve other experiments lie between these limits. While a difference of this character, which is 16.8 times its probable error, is undoubtedly significant the question immediately arises as to whether such a difference is due to differences in the blood of different rats or to a personal variation in making the measurements. A definite answer cannot be given to this question at the present time although experiments are being carried out to ascertain, if possible, which is the determining factor. As, however, this is the greatest difference obtained in twelve experiments, we may say that growing the same "pure line" in different rats probably causes significant differences in mean size, but in any case these differences are small. Even if these differences are significant there is no evidence, at present, that they are due to inherited diversities, but are simply due to differences in environment.

In regard to variability, the chief thing of interest is the fact that passage of the "pure line" from rat to rat by blood inoculation has no effect on the coefficient of variation. Take, for example, the following experiment. The coefficient of variation for total length in the "pure line" as grown in rat 116 was $2.77 \pm .13\%$. After four passages through white rats this line was grown in rat 204 where the coefficient of variation was found to be $2.60 \pm .12\%$. The difference between these two coefficients is $.17 \pm .18\%$ which certainly is not significant. Several other experiments gave similar results and an experiment is being carried out now in which the "pure line" is undergoing numerous transfers from one rat to another. The fact that passage of the pure line through rats has no effect on the coefficient of variation is in marked contrast to conditions found after passage through the invertebrate host.

Effect of Passing the "pure line" through the Invertebrate Host.—While T. lewisi can be experimentally transmitted by several species of ecto-

parasites the natural insect vectors are the rat fleas, Xenopsylla cheopis and Ceratophyllus fasciatus. In working out the life history of certain species of trypanosomes some of the earlier investigators described what they considered to be sexual phases in the life history of the parasite. Practically all of these observations are open to entirely different interpretations, so that at the present time, although a number of protozoölogists feel that there is a sexual phase in the life cycle, no such phase has been demonstrated. This is notably true in the classic researches of Minchin and Thomson[4] on the life history of T. lewisi in the rat flea. Although they looked carefully for conjugation they found no evidence of it.

While there is no evidence of sexual phenomena in the trypanosomes during passage through the invertebrate host, there is evidence that such passage exerts a profound effect on such things as acquired physiological characteristics. Gonder[5] for example found that arsenic fastness was transmitted from rat to rat but was lost by passage through the louse. Miss Robertson[6] found that strains of T. gambiense showed marked changes in their characteristics after passage through the tsetse-fly. This led this author to say, "It seems clear that the cycle in the fly as a whole, whether conjugation occurs or not, has much of the biological significance of the process."

As we have seen passage of the same "pure line" through a number of rats does not increase the variability of the "pure line." Passage through the flea, on the other hand, *invariably* increases the variability. These experiments were carried out in the following manner. Fleas which were carefully raised in the laboratory and which were known to be free from infection were allowed to bite animals infected with a given "pure line." After about a week (during which time the trypanosomes were undergoing their development in the flea) other rats were infected either by teasing up a single flea and injecting it intraperitoneally or by allowing the rat to lick up the moist feces, which is the natural mode of infection. The trypanosomes in the rat which was infected from the flea were measured on the 30th day of the blood infection. Six such experiments have been successfully carried out. The following is a fair example of all of them. The pure line in rat 105 showed a coefficient of variation of $2.80 \pm .13\%$ for total length. After passage through a single specimen of X. cheopis it was measured in rat 163. Here the coefficient of variation increased to $5.24 \pm .25\%$, a difference of $2.44 \pm .28\%$. As we have seen above, no such increase in variability has been observed during passage of the "pure line" from rat to rat. From these experiments we must conclude that the "pure line" breaks up during passage through the invertebrate host. This is analogous to the results of Jennings[7] in Paramecium after conjugation and in a recent publication Miss

Erdmann[8] believes that she has demonstrated the same type of phenomena in Paramecium after endomixis. While the increase in variability is probably due to some nuclear phenomena during the life cycle of the trypanosome in the flea, we have no method of judging, from the present data on the subject, whether it is a sexual or a reorganization process. Minchin and Thomson[4] have suggested that the peculiar effects produced by passage through the invertebrate host are connected with the transformation of the trypanosome into a crithidial stage and the reverse process, both of which always take place in the life cycle in the invertebrate host.

It is conceivable that passage of a "pure line" through the invertebrate host might cause significant changes in the mean size as well as in the variability. All of our experiments have shown this not to be true. It is true that after the "pure line" is passed through a flea there is often a difference in the mean which is significant from a statistical standpoint, but as these differences are never greater than the maximum difference given above for growing the "pure line" in different rats, we cannot ascribe the difference to any peculiar effect of the flea. It is probably due simply to the fact that the trypanosomes are measured in different rats.

Size and Variability in "wild"[1] Infections Occurring in Nature.—A number of naturally infected rats were collected from around Baltimore and Washington. Up to the present time measurements have been made of specimens from ten of these infections. While these "wild" infections do show differences in their means and a higher coefficient of variation on the average than the pure lines, one is struck with the constancy of the means and the low variability. The longest mean length (rat 221) obtained is $32.503 \pm .060$ and the shortest (rat 411) $29.093 \pm .062$ with a difference of $3.410 \pm .087$. It can be seen that the difference between the longest "wild" infection and the shortest is comparatively great and that the difference is much greater than is obtained by growing a "pure line" in different rats. The least variable "wild" infection (rat 413) showed a coefficient of variation of only $2.125 \pm .101$. This is slightly lower than any of the coefficients of the "pure lines" in the laboratory. The most variable "wild" infection (rat 65) showed a coefficient variation of $4.583 \pm .223$ The difference between the least and the most variable "wild" infection is 2.45 ± 24. The remainder of the "wild" infection measure fall between the figures given here, both as regards their means and coefficients of variation.

From these results we must conclude that while the "wild" infections occurring in nature show comparatively small differences in size, these differences are greater than can be explained by the fact that the infections are occurring in different rats. We must also conclude that most "wild" infections consist either of very few "pure lines" or a larger number

which differ *inter se* by very minute differences. In some cases, as in that of rat 413, we are almost forced to believe that a given "wild" infection is actually a "pure line." A possible explanation of these facts suggests itself when we consider the mechanism of infection with T. lewisi. As stated above the rat receives the infection from the flea. As the infected flea is feeding it deposits its feces, which contain the infective forms of the trypanosomes, on the fur of the rat. The rat then becomes infected by licking up the *moist* feces containing the infective forms. In nature it is probable that a given rat receives its infection from a single flea. This flea deposits a number of infective forms. The chances are that most of these will be killed by drying and that only a few will actually start the infection in the rat. This being so, the variability of the infections simply reflect the number of infective forms of the trypanosomes that actually start growth in the rat. In fact it is conceivable that a number of the "wild" infections are actually "pure lines."

At the present time the author is of the opinion that if we consider all the infections in nature collectively we can say that there are a large number of "pure lines" which differ among themselves but which (as is shown by work in the laboratory) are *per se* constant in size. Due to the peculiar way in which rats receive their infections, however, we find that any given infection consists of only a very few of these "pure lines."

Conclusions.—At the present phase of the work the following conclusions may be drawn: While growing the same "pure line" in different rats may cause significant differences in the mean size, these differences are small. The differences in the mean are never greater when the "pure line" is grown in different species of rats than when it is grown in different individuals of the same species. Passage of the "pure line" from rat to rat is not followed by any significant changes in the coefficient of variation. In marked contrast to this it is found that passage of the "pure line" through the flea is invariably followed by a significant increase in the coefficient of variation which is interpreted as showing that the "pure line" breaks up into heritably diverse lines following such passage. Although passage through the flea has such a marked effect on the variability it has no significant effect on the mean size. "Wild" infections occurring in nature exhibit differences in their mean sizes which are greater than can be explained by the fact that they occur in different rats. The coefficients of variation of "wild" infections are, on the average, higher than for the "pure line." The lowest of these coefficients, however, is no greater than the coefficient of variation for the "pure line" and even the highest can be explained on the assumption that the "wild" infection consists of only a very few "pure lines." It seems probable that there occur, in nature as a whole, a large number of

"pure lines" which differ among themselves but are *per se* constant in size, but, due to the peculiar way in which rats receive their infections, a given infection consists of only a very few of these lines. In fact, a large number of "wild" infections are probably actual "pure lines."

[1] Throughout this work the term "pure line infection" is used to designate an infection, the trypanosomes of which have all arisen from a single organism. A given "pure line" may either have been started from a single trypanosome, or it may have been subinoculated from such an infection. The term "wild infection" designates an infection as found in nature.

[2] These PROCEEDINGS, **7**, 1921 (138–143).

[3] The mean sizes in this report are all in microns.

[4] Minchin, E. A., and Thomson, J. D., *Quart. J. Microsc. Sci.*, **60**, *N. S.*, 1915 (463–692).

[5] Gondor, R., *Centralbl. Bakt., etc.*, I abt., Originale, **612**, 1911 (102–113).

[6] Robertson, M., *Proc. Roy. Soc.*, (B) **85**, 1912 (241–248).

[7] Jennings, H. S., *J. Exper. Zool.*, **11**, 1911 (1–134); *Ibid.*, **14**, 1913 (270–391).

[8] Erdmann, R., *Archiv. Entwicklungsmech. Organ.*, **46**, 1920 (85–148).

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*NOTE ON MOVING EQUILIBRA**

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A number of previous publications[1] have been devoted to the study, from various angles, of a material system evolving in accordance with a system of differential equations

$$dX_i / dt = F_i(X_1, X_2, \ldots; A_1, A_2, \ldots; P; Q) \qquad (1)$$

where the X's denote the masses of the several components of which the system is built up; the A's are parameters introduced by any equations of constraint to which the X's may be subject;[2] the parameters P include geometrical constraints (volume, area, topography) and also other quantities serving to define the state of the system (temperature, etc.). The Q's define the character of the several components or species.

The discussion has hitherto been restricted to the case that the parameters A, P, Q remained constant during the transformations taken in view. A complete discussion of the evolution of systems of the kind referred to must include also the consideration of changes in these parameters.

Such changes may be grouped under three heads:

1. Changes of a perfectly general character. A study of these would resolve itself into a discussion, on a general basis, of a system of differential equations of the form

$$dX_i / dt = F_i(X_1, X_2, \ldots, t) \qquad (2)$$